



Developmental plasticity in an unusual animal: the effects of incubation temperature on behavior in chameleons

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Abstract

The thermal environment within a reptile nest can affect the phenotypic traits of hatchlings, and hence (potentially) their fitness. Research on this topic needs to measure phenotypic traits relevant to the species involved; and hence, studies on unusual species need to measure unusual traits. We investigated the effects of two incubation temperatures on the morphology and behaviour of hatchling veiled chameleons (*Chamaeleo calyptratus*). Colder-incubated eggs exhibited a longer incubation period, but produced larger faster-growing hatchlings. Incubation treatment also affected a chameleon's activity level and its unique foraging tactics. Cold-incubated animals were more sedentary, caught prey faster, and extended their tongues farther to reach prey than did their warm-incubated counterparts. Thus, the fitness costs of low temperatures in the nest (slow development, and thus late hatching) may be offset by incubation-derived enhancements in hatchling growth rates and foraging abilities.

Keywords

phenotypic plasticity, nest temperatures, foraging behaviour, lizard.

1. Introduction

Ambient temperatures not only exert powerful direct effects on many aspects of organismal function ([Hoffmann & Sgrò, 2011](#)), but also can shape developmental trajectories ([Sinervo & Adolph, 1989](#); [Pétavy et al., 1997](#)). Most obviously, unfavourably high or low temperatures can curtail activity levels, feeding rates and thus growth rates, so that an individual's body size is influenced by its prior thermal experiences ([Autumn & Nardo, 1995](#);

Lance, 2003). Developmental effects of ambient temperature can also influence many other traits. For example, in species where embryonic life is spent in an external nest, the thermal regimes experienced by eggs within that nest can powerfully affect the phenotypic traits of animals that emerge from those eggs (Booth, 2006). An extensive literature on reptiles illustrates pervasive effects of incubation temperature on traits such as hatchling body size and shape, growth rates, behavior, and cognitive abilities (Burger, 1991; Allsteadt & Lang, 1995; Rhen & Lang, 1995; Elphick & Shine, 1998; Amiel & Shine, 2012). Such norms of reaction may have played an important role in life-history evolution within this group (Warner & Shine, 2008; Shine, 2013).

In order to clarify the adaptive significance of phenotypically plastic responses to incubation conditions, we need to manipulate the embryos' environment and measure the consequences for fitness-relevant phenotypic traits. Squamate reptiles have become popular model organisms for such studies, and researchers have explored how incubation conditions affect hatchling phenotypes across many reptilian taxa. Nonetheless, the suite of phenotypic traits examined in this respect has been relatively narrow, with most studies restricted to incubation effects on the morphology and locomotor performance of hatchlings (Burger, 1991; Damme et al., 1992; Janzen, 1993; Elphick & Shine, 1998; Du & Ji, 2003; Andrews, 2008). Although those traits are likely to be highly relevant to individual viability (and thus fitness) in many taxa, no single suite of traits will be universally relevant to all species. The spectacular diversity of squamates (Pianka & Vitt, 2003) means that a trait such as locomotor speed is far more important for some taxa than for others (depending on their habitat use, antipredator and foraging tactics); and that even within the broad category of locomotor ability, some species may depend upon high speeds on flat ground whereas others rely upon an ability to rapidly climb thin branches, or cling onto a surface (Losos et al., 1993). In order to understand how phenotypically plastic responses to incubation conditions affect organismal function, we need to match the variables measured to the organism's biology; and only by incorporating 'unusual' taxa, can we hope to clarify broad patterns in the role of developmental plasticity in squamate ontogeny.

Arguably, chameleons are among the most bizarre reptiles (and indeed, vertebrates). In striking contrast to other lizard taxa, chameleons display

a range of morphological and behavioural traits linked to a specialized arboreal lifestyle and ambush foraging tactics (Schmidt, 2001). For example, chameleons ballistically extend their tongue up to twice the length of their body to capture prey (de Groot & van Leeuwen, 2004) while limiting movement and thus, remaining inconspicuous to potential predators. To detect conspecifics, predators, and prey in the three-dimensional space they inhabit, chameleons have independently-operating eyes; and to remain hidden yet send social signals without needing to move, these lizards are able to rapidly change from colours that closely match their surroundings to those of brilliant contrast that they use in social communication (Cuadrado et al., 2001; Stuart-Fox & Moussalli, 2008).

These distinctive traits suggest that some conventional ‘performance’ measures (such as ability to sprint rapidly on flat ground) may be largely irrelevant to chameleon biology. Instead, a chameleon’s viability may depend upon factors such as its ability to feed while remaining sedentary — and thus, fitness may be highest for individuals that move about very little, but are able to propel their tongues long distances, and accurately, when prey venture within range. We explored incubation effects on these traits in the veiled chameleon, *Chamaeleo calypttratus*. This large and spectacular species is common in the commercial pet trade, but its biology in nature is virtually unstudied because of logistical obstacles to research in its native range. Hence, most of what we know about veiled chameleons comes from studies of captive animals (Barnett et al., 1999; Kelso & Verrell, 2002; Andrews, 2004, 2008).

2. Methods

2.1. Study species

Chamaeleo calypttratus is a medium-sized arboreal oviparous diurnal chameleon distributed throughout western Yemen and southern Saudi Arabia, in habitats ranging from humid low coastal plains to mountain slopes (De Vosjoli & Ferguson, 1995). We chose *C. calypttratus* as our study organism for several reasons. First, females lay their eggs shortly after fertilization, when the offspring is in the gastrula stage (Andrews, 2004), allowing us to control the incubation environment for nearly the entirety of development. In most oviparous squamates, females retain eggs in utero for at least a quarter of the total embryonic development, so that post-oviposition manipulations

of incubation environment affect a smaller proportion of the total embryonic phase ([Shine, 1983](#); [Blackburn, 1995](#)). Second, these animals are easily sexed at hatching because males have a ‘spur’ on the foot whereas females do not ([De Vosjoli & Ferguson, 1995](#)). Third, females produce clutches of up to 60 eggs, facilitating split-clutch experimental manipulation. Fourth, laboratory experiments have shown that incubation temperature does not affect sex ratios of offspring, but may modify other phenotypic traits ([Andrews, 2005, 2008](#)). Fifth, these animals grow fast, attain maturity in a few months, and typically have only brief lifespans ([Schmidt, 2001](#)); thus, relatively short-term studies can capture a significant fraction of an individual’s lifespan.

2.2. *Behavioural trials and husbandry*

In September, 2011 we collected three clutches ($N = 100$; 43, 27 and 30 eggs per clutch) of recently-laid (<12 h post-laying) eggs from a captive population of chameleons at University of Sydney and placed the eggs into either ‘cold’ (25°C) or ‘warm’ (28°C) constant-temperature incubators for the entire 6 month incubation duration. Individual egg containers contained moist (−200 kPa) vermiculite, following [Andrews & Donoghue \(2004\)](#). Moisture is not considered in the experimental analysis because previous studies have shown its effects on hatchling phenotype to be negligible compared to those of temperature treatment (in *C. calypratus*: [Flatt et al., 2001](#); [Andrews & Donoghue, 2004](#); [Warner et al., 2012](#)). To reduce bias from maternal effects on hatchling traits, clutches were split randomly between the two incubation conditions and after hatching, males and females were randomly allocated to either isolated or group rearing enclosures. We maintained young lizards either in isolated conditions (in opaque plastic containers 270 × 190 × 100 mm) or in groups of four (in enclosures about four times larger than the isolated enclosures: 620 × 370 × 120 mm). A thermal gradient within each cage (from 28°C to >35°C heat source) allowed for behavioral thermoregulation for 9 h per day in both groups; for the rest of the diel cycle the room temperature was 26°C. All enclosures contained artificial plants and dowel sticks for perch and roost sites, providing animals with the opportunity to engage in their natural arboreal behaviour. Animals received ad libitum food (calcium-dusted crickets and mealworms) every day and were watered twice a day. Rearing conditions (isolated vs group) were also included in our analyses to control for any effects ([Ballen et al., 2014](#)). After the experimental period we distributed study animals to Zoo and Aquarium

Association-accredited zoos or other research institutions, as stated in the University of Sydney animal ethics agreement. We placed animals in cloth pillowcases to move them between sites in order to minimize stress. All experimental procedures were approved by University of Sydney Animal Ethics Committee (L04/4-2012/3/5678).

The influence of incubation temperatures on development was assessed by recording rates of egg survival to hatching, hatchling morphology, growth rate and behaviour (foraging tactics and ability, and general activity). At hatching, and at 15-day intervals thereafter up to 60 days, we recorded mass, snout-vent length (SVL) and tail length (TL). Foraging experiments were conducted as soon as the young lizards began to feed (from 3–5 days old), and at 30 days and 60 days of age. For each animal at each age group, five foraging trials were performed on five consecutive days, resulting in a total of 769 total trials across all individuals. If an animal did not eat, we waited 24 h and then repeated the foraging trial, making note of the number of aborted trials per individual.

Animals were transferred from their cage to the experimental enclosure as fast as safely possible, within 30 s. They were placed under an opaque hide in the middle of a sterile empty enclosure (270 × 190 × 100 mm) and allowed five minutes to settle before the hide was lifted and they were exposed to three crickets moving in the container. Hatchlings were tested using crickets that were <3 mm, and juveniles (30 days and 60 days) tested with crickets that were 3–5 mm in length. All foraging trials were video-recorded for subsequent behavioural analysis; the trial was stopped when the observer witnessed an animal successfully capture a cricket on the camera monitor. The observer remained out of view of the animal for the duration of the trial. A grid (5 mm graph paper) below the transparent container enabled us to estimate the following from the video: (1) distance of tongue extrusion, from the tip of the animal's nose to the prey item, (2) movement, the distance travelled by the animal prior to an attempt to capture prey, (3) latency of approach, or the duration of time from when the focal animal was exposed to the crickets until it attempted to seize one of them using ballistic tongue movement, (4) number of 'misfires' (unsuccessful capture attempts) and (5) number of trials with no feeding response. The day before animals began foraging trials, we video-recorded them (without prey) in the same container to measure their activity levels (distance travelled during a ten-minute period, using the grid lines to quantify displacements). Before all

behavioural trials, heat lamps were turned off so animals could equilibrate at the 26°C ambient room temperature for 20 min.

2.3. Analysis

In all analyses we included two factors: incubation temperature (25 vs. 28°C) and rearing condition (isolated vs. group). For the traits we consider here, there were no interactions between rearing conditions and other variables (such as incubation temperature), so we deleted these effects and recalculated the models; we do not report these rearing-condition effects in the present paper (but see Ballen et al., 2014). Instead, we focus on the main effects of incubation temperature, sex, age, and their interactions. In preliminary analyses, clutch of origin did not significantly influence the hatchling responses to incubation conditions (all interactions clutch \times treatment $p > 0.05$). However, we also included mother ID and individual ID in the analyses as random factors. Random effects were tested by running PROC MIXED twice, once for the full model and once for the reduced model without the random factor being tested, and then subtracting the corresponding values of -2 times the log likelihoods. The resulting statistic, which tests whether the full model is necessary beyond the reduced model, was tested against a chi-square distribution with one degree of freedom, equal to the difference in the number of covariance parameters between both models (SAS/STAT, 9.1, User's Guide, Vol. 4, p. 2741; SAS Institute, Cary, NC, USA).

To examine incubation duration, morphology, and growth rate, we used a mixed-model procedure in SPSS 20.0. Specifically, we evaluated the effect of incubation temperature (25°C vs. 28°C) and sex on incubation duration (days spent in incubation treatment until hatching), hatchling size (mass, SVL and TL) and hatchling growth (mass, SVL and TL per day) over the first month and second month of age. Egg mass was included as a covariate for all analyses. To test whether egg viability was affected by incubation temperature, we used a Pearson chi-square test for independence.

To explore differences in foraging behaviour and activity between treatments, we ran mixed model analyses entering offspring ID and dams as random variables with incubation temperature, sex, age, and sequential trial number (1–5) as fixed factors. We also included possible interactions in the analyses. Degrees of freedoms (Walker, 1940) for the GLMMs were calculated using the DDFM = KENWARDROGER option in SAS 9.3. This approximation involves inflating the estimated variance–covariance matrix

of the fixed and random effects by the method proposed by [Prasad & Rao \(1990\)](#) and [Harville & Jeske \(1992\)](#). Satterthwaite-type degrees of freedoms are then computed based on this adjustment. This method generally performs best in GLMMs and we therefore follow the advice by ([Bolker et al., 2008](#)). This also reduced the AIC value from >9000 to 2358 compared to the same model using the containment method (default in SAS Proc Mixed). We retained significant covariates, and thus log transformed body mass was included as a covariate in our analyses of latency to strike, and tongue extrusion distance; and latency to strike was included as a covariate in the analysis of distance moved during a trial (SAS/STAT, 9.1, User's Guide, Vol. 4).

3. Results

3.1. Incubation duration and hatching success

The duration of incubation was strongly affected by incubation temperature, with cold-incubated animals emerging later (mean = 253.40 days, SE = 0.39) than warm-incubated animals (mean = 227.49 days, SE = 1.29; see Table 1). Incubation duration also was longer for males than for females (female mean = 239.30, SE = 1.18; male mean = 246.70, SE = 0.85), and increased with egg mass (Table 1). A Pearson chi-square test for independence revealed that embryo survival was affected by incubation treatments. In the cold temperature treatment, 76% of eggs ($N = 51$) survived until hatching, while in the warm temperature treatment, 53% survived until hatching ($N = 49$; $\chi^2 = 6.02$, $df = 1$, $p = 0.014$).

3.2. Morphology and growth rate

A hatchling chameleon's morphology and daily growth rate over the first two months were affected by the animal's prior incubation temperature and its sex ($N = 52$; Table 1, Figure 1). Cold-incubated lizards gained mass (but not SVL) faster than their warm-incubated siblings in the first month of growth, and females grew faster than males in the second month of growth (Table 1, Figure 1a, b). Females also grew longer tails in the second month of development (Table 1, Figure 1e, f). Heavier eggs did not produce heavier hatchlings, but tail length was marginally higher in animals produced from heavier eggs, whereas neither SVL nor mass showed any such pattern (Table 1).

Table 1.

Results from mixed-model statistical analyses to examine the effects of incubation temperature (25 vs. 28°C), sex, and their interaction on incubation duration, hatching size (mass, SVL and TL), and hatching growth (mass, SVL and TL per day) of veiled chameleons during the first month and second month of life.

Trait	Main effect of incubation temperature	Main effect of sex	Temp × sex interaction	Egg mass
Incubation period (days)	$F_{1,49} = 58.88, p < 0.001^*$	$F_{1,49} = 5.78, p = 0.019^*$	$F_{1,49} = 1.06, p = 0.312$	$F_{1,25} = 5.88, p = 0.023^*$
Overall morphology				
Mass (g)	$F_{1,264} = 8.39, p = 0.004^*$	$F_{1,264} = 5.83, p = 0.016^*$	$F_{1,264} = 1.75, p = 0.188$	$F_{1,264} = 0.00, p = 0.988$
Snout-vent length (mm)	$F_{1,264} = 1.83, p = 0.177$	$F_{1,264} = 2.14, p = 0.144$	$F_{1,264} = 0.77, p = 0.382$	$F_{1,264} = 0.00, p = 0.974$
Tail length (mm)	$F_{1,48} = 0.01, p = 0.934$	$F_{1,48} = 0.00, p = 0.969$	$F_{1,48} = 1.22, p = 0.274$	$F_{1,49} = 4.16, p = 0.047^*$
1st month growth				
Mass per day (g)	$F_{1,43} = 7.81, p = 0.008^*$	$F_{1,43} = 0.00, p = 0.978$	$F_{1,43} = 0.69, p = 0.411$	$F_{1,43} = 0.44, p = 0.509$
Snout-vent length per day (mm)	$F_{1,43} = 0.34, p = 0.566$	$F_{1,43} = 0.01, p = 0.916$	$F_{1,43} = 1.77, p = 0.190$	$F_{1,43} = 0.24, p = 0.626$
Tail length per day (mm)	$F_{1,43} = 0.32, p = 0.576$	$F_{1,43} = 0.48, p = 0.494$	$F_{1,43} = 0.45, p = 0.508$	$F_{1,43} = 0.57, p = 0.455$
2nd month growth				
Mass per day (g)	$F_{1,42} = 0.12, p = 0.732$	$F_{1,42} = 6.81, p = 0.012^*$	$F_{1,42} = 0.16, p = 0.694$	$F_{1,42} = 2.43, p = 0.126$
Snout-vent length per day (mm)	$F_{1,42} = 0.39, p = 0.537$	$F_{1,42} = 1.54, p = 0.221$	$F_{1,42} = 3.59, p = 0.065$	$F_{1,42} = 5.70, p = 0.022^*$
Tail length per day (mm)	$F_{1,42} = 1.65, p = 0.206$	$F_{1,42} = 7.38, p = 0.010^*$	$F_{1,42} = 0.23, p = 0.636$	$F_{1,42} = 1.65, p = 0.206$

Egg mass was included as a covariate for all analyses.

* Significant value ($p < 0.05$).

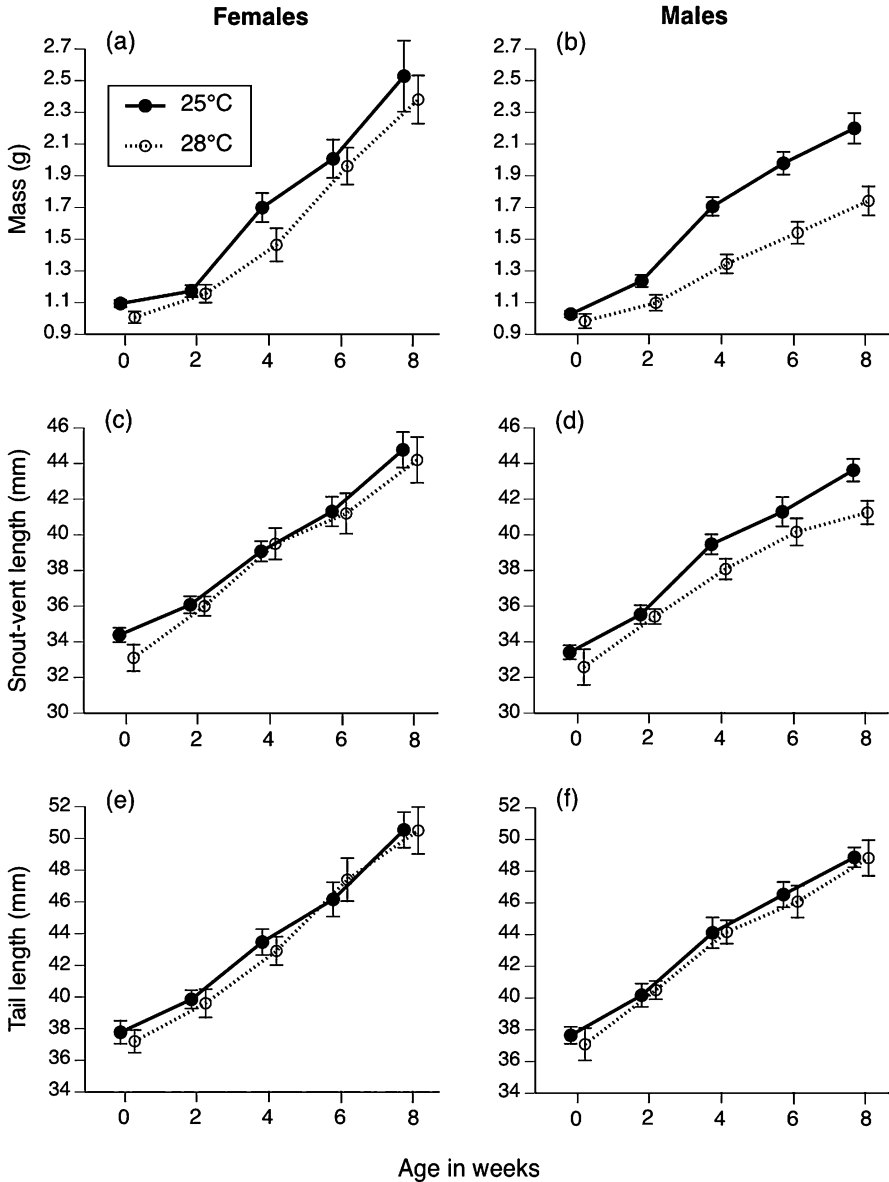


Figure 1. Effects of incubation temperature on mass, snout-vent length and tail length of female (a, c, e) and male (b, d, f) juvenile *Chamaeleo calypratus* over the first two months of posthatch life.

3.3. Foraging tactics and abilities

Incubation temperature affected a young lizard's foraging behaviour, with cold-incubated individuals stretching their tongues farther than did warm-incubated conspecifics (Table 2, Figure 2a). This incubation effect was not a secondary consequence of body-size differences: a lizard's body mass did not significantly affect the length that it extruded its tongue during prey-capture attempts ($F_{1,677} = 1.69$, $p = 0.19$). Cold-incubated lizards also took less time to capture a prey item after it was dropped into their enclosure (Figure 2b). This latency was also affected by age and mass, with older and larger lizards capturing prey sooner than did younger smaller individuals (Age: $F_{2,673} = 19.96$, $p < 0.01$; Mass: $F_{1,673} = 3.86$, $p = 0.05$). The mean distance that a lizard traveled before it attempted to catch a cricket also differed between incubation groups, with cold-incubated animals moving less than warm-incubated animals (Table 2; Figure 2c). However, that difference was due to the greater latency values for warm-incubated individuals; when we included 'time prior to launching an attack' as a covariate, the treatment effect on distance moved was no longer significant ($F_{1,242} = 0.69$, $p = 0.41$). Thus, lizards in the two groups moved at similar rates per unit time, but warm-incubated lizards covered a greater absolute distance because they delayed launching a prey-capture attempt. The number of ballistic tongue

Table 2.

Results from mixed-model statistical analyses to examine the effects of incubation temperature on aspects of foraging behaviour and activity of veiled chameleons at hatching (3–5 days), and at 1 month and 2 months of age.

Behavioural variable	Covariate	Main effect of incubation temperature		
		df	<i>F</i>	<i>p</i>
Foraging				
Latency to strike (s)	Mass (g)	1, 40	3.96	0.053
Tongue extrusion (mm)	Mass (g)	1, 44	17.84	<0.001*
Distance moved during trial (mm)	Latency (s)	1, 49	19.31	<0.001*
No. of misfires	–	1, 18	4.16	0.056
No. of trials with no feeding response	–	1, 39	0.77	0.390
Activity (mm)	–	1, 93	10.82	0.001*

* Significant value ($p < 0.05$).

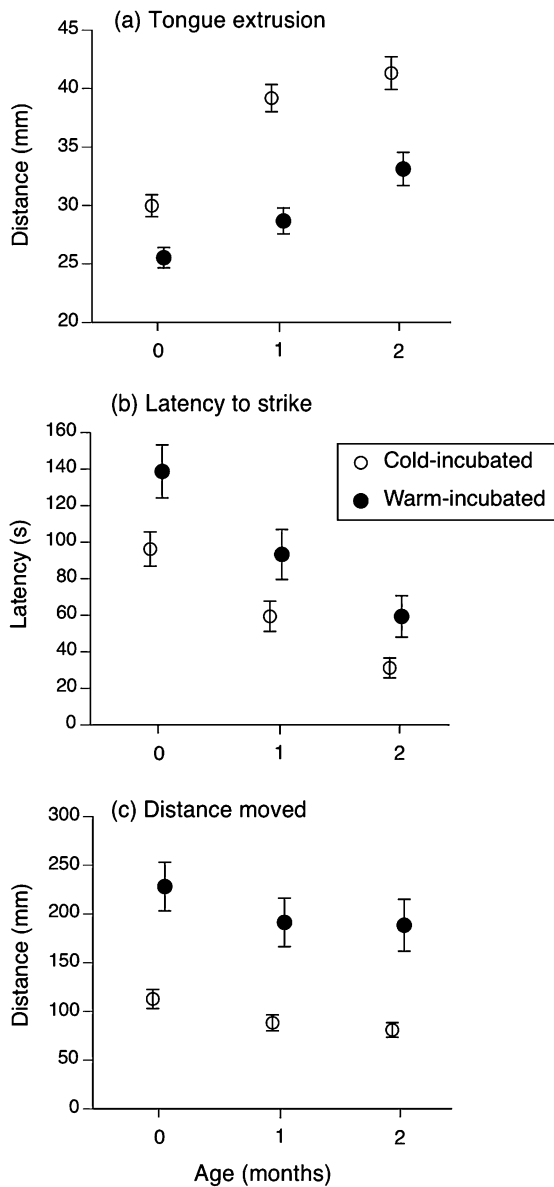


Figure 2. Foraging performance of veiled chameleons (*Chamaeleo calytratus*) incubated in either 'cold' (open circles) or 'warm' (filled circles) temperatures. Performance parameter means (± 1 SE) are presented from trials run at hatching (3–5 days), 1 month, and 2 months of age.

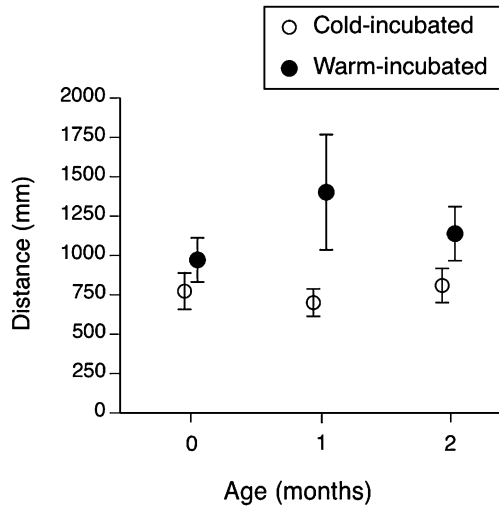


Figure 3. Activity, or the distance individuals traveled in a ten minute period, of veiled chameleons (*Chamaeleo calyptratus*) incubated in either ‘cold’ (open circles) or ‘warm’ (filled circles) temperatures. Activity means (± 1 SE) are presented from different ages at which experimental trials were conducted.

misfires during foraging tended to be higher in cold-incubated lizards, but this difference did not attain statistical significance ($F_{1,18} = 4.16$, $p = 0.06$; Table 2). Incubation temperature did not affect propensity to feed (i.e., the proportion of trials with no feeding response did not differ significantly between groups: Table 2).

3.4. Activity levels

In all three age groups that we tested (at hatching, 1 month, and 2 months of age), warm-incubated lizards moved around more than did their cold-incubated siblings ($F_{1,93} = 10.82$, $p < 0.01$; Figure 3).

4. Discussion

Incubation temperature affected egg survival and hatchling phenotypes in our chameleons, as has been reported previously for a diverse array of oviparous animals (in birds: [DuRant, 2013](#); in reptiles: [Booth, 2006](#)). Although the effects of incubation temperature on hatchling phenotype have been researched extensively, most work on reptiles has focused on ‘typical’ (e.g., terrestrial) taxa, with little attempt to match measures of phenotypic performance to

unique aspects of the study species. To incorporate a diversity of taxa, we will need to measure a diversity of fitness-related traits (i.e., that are relevant to those species) and hence, will need novel ways to assess the most important variables. This customized approach will better reflect the biologically significant effects of environmental variation in temperature on hatchling phenotypes and developmental trajectories. For example, although speed in sprinting or climbing may occasionally benefit a chameleon ([Andrews, 2008](#)), foraging skills (e.g., response time and length of tongue extrusion while foraging) and behaviours that minimize detectability to predators (e.g., lack of movement) are more likely to affect a young chameleon's growth and survival. Below, we discuss the effects of incubation treatment, and whether or not those effects may be adaptive for *C. calypttratus*.

4.1. Incubation effects

Based on a total of 769 observations of prey capture attempts from 56 individuals, we found that cold-incubated hatchlings took less time to capture prey, protruded their tongues farther relative to their body mass, and moved less in order to capture prey. Cold-incubated animals also moved less than their warm-incubated counterparts during the 'activity level' experiments, in which we measured the distance travelled during a ten-minute period. This impact was especially pronounced at one month of age, suggesting a complex ontogenetic progression in incubation-induced effects. Although all animals were offered equal amounts of food per day, cold-incubated animals were larger and grew faster. For a sit-and-wait (ambush) predator like the chameleon, an ability to seize prey from a considerable distance, without moving the body, may maximise foraging success while minimizing energy expenditure and exposure to predators. Our data support that scenario — for example, animals with shorter latency to prey capture during foraging experiments (the cold-incubated lizards) also grew more rapidly during the first two months of life. An alternative explanation may be that warm-incubated animals respond differently to the stress of captivity and handling (as reported by [Trnik et al., 2011](#)), and what we observed were the products of elevated stress levels.

Because the size of the tongue pad scales with tongue length ([Anderson et al., 2012](#)), our lizards with longer-protruded tongues (cold-incubated) may also have been able to seize larger prey items. [Anderson et al. \(2012\)](#) reported that smaller individuals within and among species have proportionately longer tongues than larger individuals, whereas we found no such effect

(based on the maximum length extruded in an attempt to capture prey). However, our data relate to extrusion length, whereas Anderson's (2012) study was based on direct measurements of dead animals. That difference in methods may explain the divergence between our results. The patterns that we found may reflect lizard behaviour (the proportion of its maximum tongue length extruded) rather than actual tongue length. The former scenario suggests an influence of incubation temperature on cognition rather than tongue morphology.

Overall, our results suggest that warm-incubated animals benefit from an early hatching date but at the cost of a less effective phenotype, whereas cold-incubated animals hatch later but emerge with a superior phenotype. The relative fitness outcomes of that trade-off are unclear, and presumably are affected by specific aspects of chameleon natural history. For example, if hatchlings disperse soon after they emerge from the egg, then a cold-incubated chameleon may not be likely to encounter (and, thus, have to compete with) older (and, therefore, larger) conspecifics when it emerges. The seasonal timing of oviposition, and the availability of various thermal regimes at potential nest sites, also may play major roles. For example, slower development may permit eggs that are laid in cold seasons to hatch in more favourable (warmer) times of year. The lack of field studies on our model species means that we cannot test such speculations; all we can do is point to the possibility that the impacts of nest temperatures on hatchling fitness in this system may be reduced by a trade-off between early emergence and superior performance.

4.2. Sex differences

Female chameleons hatched after a shorter incubation duration than did males, and females grew faster than males (Table 1, Figure 1). Could such differences be adaptive, because of sex differences in the degree to which early hatching and rapid growth affect lifetime reproductive success? Plausibly, female chameleons that hatch early and grow rapidly may thereby avoid sexual harassment. Male chameleons relentlessly pursue and court females, despite conspicuous colour signals indicating female non-receptivity (in common chameleons: Cuadrado, 2000). Female reptiles also benefit from rapid attainment of larger size because of higher fecundity and earlier maturation ([Bonnet et al., 2001](#); [Litzgus et al., 2008](#); [Beldade et al., 2012](#)).

The critical importance of suitable nest temperatures for oviparous reptiles has obvious implications for the vulnerability of these animals to change in

global temperatures ([Janzen, 1994](#); [Mitchell et al., 2008](#)). To evaluate the generality of impacts of thermal shifts on reptile biology, we need research on a wide range of species. Importantly, that increase in taxonomic breadth means that we also need to expand our methodologies, to tailor the traits that we monitor to the biology of our study species. The importance of specific traits for fitness variation among individuals undoubtedly differs among species. Measurements of hatchling morphology and running speeds have been the staple currencies in this research field for many years, but we need to take a broader perspective if our studies are to encompass a higher proportion of the biological diversity that is exhibited by oviparous vertebrates.

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